Sortase A

Week 14

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 PCR of truncated SortA for Gibson Assembly

Responsible

Aman Mebrahtu

Protocols used

PCR Amplification (Q5)

Modifications and comments to protocols

No modifications were made to the protocol.
Primers used: Forward - $SortA_Forward_1$, Reversed - $Sort_GA_R$.
Annealing temperature was set to 58°C.

PCR of truncated Sortase A to ad suitable overhangs upstream and downstream the coding sequence

Experimental Set Up

Samples: Sortase DNA 1.2, Sortase DNA 2.1, Sortase DNA 2.2

Table 1: Sample set up.

Sample No.	Source Sample	Sample Name
1	Sortase DNA 1.1	SortA-GA-1
2	Sortase DNA 2.1	SortA-GA-2
3	Sortase DNA 2.2	SortA-GA-3
4	-	Negative control

Table 2: PCR Reaction Set-up for all samples.

Component	25 μl Reaction	Final Concentration
Q5 2X High-Fidelity Master Mix	12.5	1X
10 μM Forward Primer	1.25	0.5 μM
10 μM Reverse Primer	1.25	0.5 μM
Template DNA	1	
Water	9	

Results and Conclusions

Electrophoresis of samples generated a gel with observable bands at a desired size just below 500 bp. In conclusion the PCR was successful.

Table 3: Nanodrop Concentrations of samples after PCR amplification.

Sample	Concentration [ng/μl]
SortA-GA-1	268.1
SortA-GA-2	257.3
SortA-GA-3	217

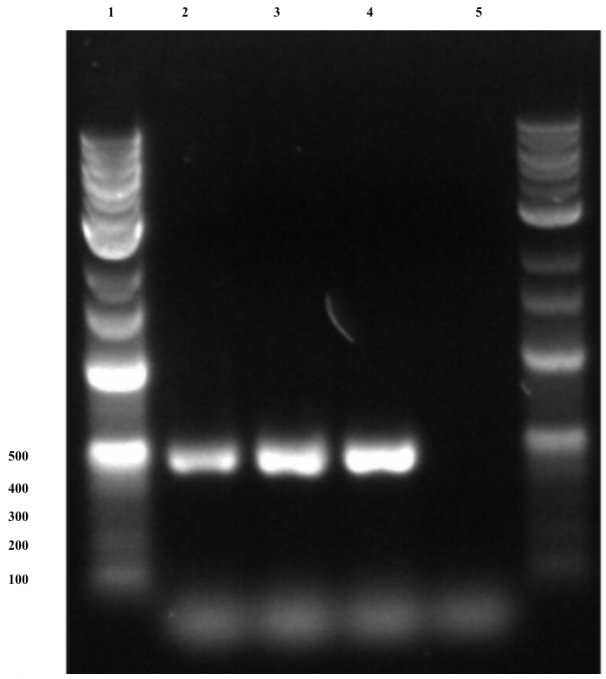

Figure 1: Gel electrophoresis results in 1 % agarose, at 110 V. (1) DNA Ladder (2) SortA-GA-1 (3) SortA-GA-2, (4) SortA-GA-3, (5) Negative control. A band for all samples of the expected size around 500 bp can be observed.

Discussion and Troubleshooting

The PCR was successful and Subsequent step will aim to perform Gibson assembly of the PCR samples with GB1-Solubility tag and vector Backbone pSB1C3.

2 Gibson assembly - Trial 1

Responsible

Aman Mebrahtu

Protocols used

Gibson Assembly

Modifications to protocols

Experimental Set Up

Sample 1 and 2 from previous experiment were used for the calculations to perform a Gibson assembly given that they had the highest concentration. The table below summarizes the sample set-up.

Table 4: Sample set-up.

Sample	Sample Name	Expected size [bp]	Comment
1	GA-1	2930	
2	GA-2	2930	
3	SortA-GA-1	523.00	Comperative
4	GB1	207.00	Compertaive
5	Negative control	-	-

Table 5: The experimental set-up for Gibson assembly. Volume of each component for the two sample mixtures GA1 and GA2 are described in the table.

	2-3 Fragment Assembly
Total Amount of Fragments	GA1 = 3.55 (GB1) + 2.1 (SortA) + 3.23 (BB) = 8.88 μl
0.5 pmoles	GA2 = 3.55 (GB1) + 2.2 (SortA) + 3.23 (BB) = 8.98 μl
Gibson Assembly Master Mix (2X)	10 l
Deionized H2O	GA1 = 10-9.65 = 1.12 l GA2 = 10-9.76 = 1.02 μl
Total Volume	20 μl

Reaction set-up for GA1 was performed, due to limited amount of Gibson assembly Master Mix

Table 6: Summary of calculations and volumes used for each fragment in the assembly.

Sample	Concentration [ng/μl]	Concentration [pmole/μl]	Desired pmole	Ratio	Size bp	Mw [Da] [g/m
pSB1AC3	25	0.0172	0.056	1:1	2200	1452000
SortA-GA-1	268.10	0.7767	0.167	1:3	523	345180
SortA-GA-2	257.30	0.7454	0.167	1:3	523	345180
GB1	10	0.0783	0.278	1:5	207	136620

Results and Conclusions

Band of interest, which should be larger than the Backbone itself cannot be observed on the gel. The assembly was not successful.

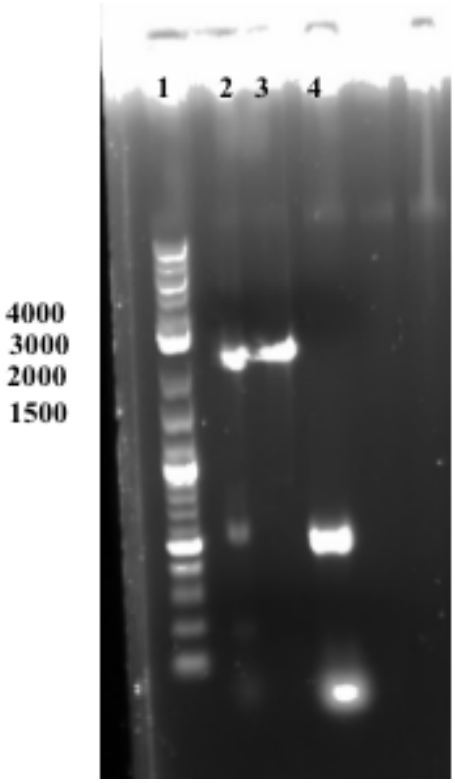

Figure 2: Gel electrophoresis results in 1 % agarose, at 110 V. (1) DNA Ladder (2) GA1 (3) pSB1C3, (4) SortA-GA-1

Discussion and Troubleshooting

The assembly failed most likely due to an imbalance in the molar ratios of fragments where the undiluted sample of SortA-GA-1 was used. Second trial of Gibson assembly to performed, using the diluted version of SortA-GA-1.

3 Gibson assembly - Trial 2

Responsible

Aman Mebrahtu

Protocols used

Gibson assembly

Modifications to protocols

No modifications were made to the protocol.

Experimental Set Up

Exact same set-up was sued for trial 2 as in the first trial.

Sample Calculation

Calculations were repeated in the same manner as in trial 1. Diluted version of SortA-GA-1 was used.

Results and Conclusions

A faint band, in the well loaded with the assembled product, can be observed slightly above the the band for the backbone thus indicating a possible successful Gibson assembly, but no definitive conclusions can be drawn.

Discussion and Troubleshooting

No definitive conclusion can be drawn regarding the Gibson assembly. To confirm whether the assembly was successful, the product will be transformed into E.coli TOP 10 and BL21 strains, expecting colony growth under antibiotic selection (chloramphenicol).

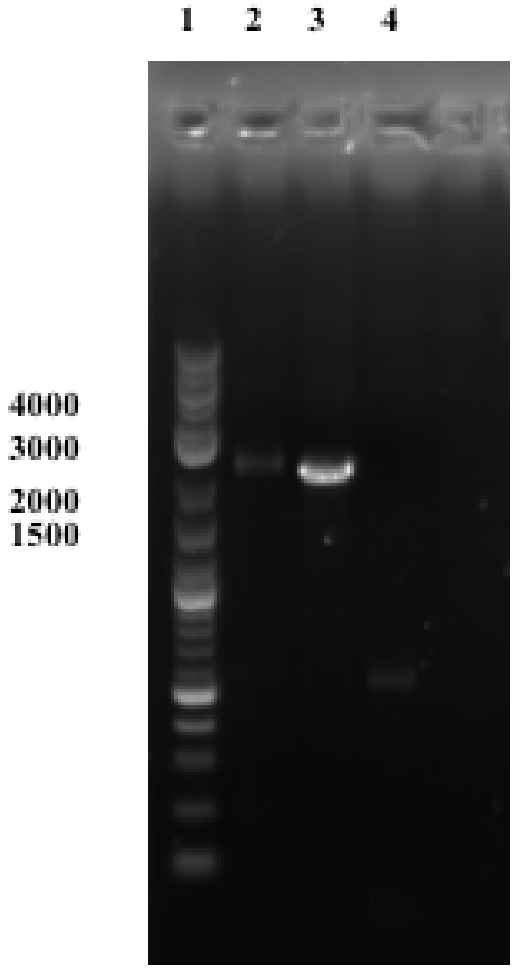

Figure 3: Gel electrophoresis results in 1 % agarose, at 110 V. (1) DNA Ladder (2) GA1 (3) pSB1C3, (4) SortA-GA-1